IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/564,788
Applicant	:	HUMMEL et al.
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Examiner	:	Ronald T. Niebauer
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DECLARATION

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Heiko Hawlisch, declare as follows:

1. I have received the following academic degrees which are relevant to the subject matter of the present application: Dr. rer. nat. (Leibniz Universität Hannover, Germany).

2. I am a specialist in pharmacology and assay development. I have 3 years of experience practicing in this field. I currently hold the position of Senior Scientist in the department of Lead Discovery Biology.

3. I understand that the subject application is directed to a compound which is a C5a receptor antagonist. I understand that C5a is a protein generated upon complement activation and that a C5a receptor antagonist would be useful in treating, for example, inflammatory diseases.

4. I understand that the Examiner has cited U.S. Pat. No. 5,387,671 (Kawai) as rendering the claimed compound obvious. I understand that Kawai is directed to C5a receptor modulating compounds. I understand that the Examiner argues that Kawai discloses compounds that are structurally similar to the claimed compound, specifically the compound in Kawai example 171.

5. The compound of Kawai example 171 was synthesized and compared to the claimed compound of the subject application using in vitro testing. This testing was performed under my direction and control.

6. A brief description of the assay used for comparison follows:
Determination of the IC50 value in an enzyme release assay: Basophilic leukemia cells from rats (RBL), which express human C5aR (CD88), were cultured in DMEM with 10% fetal bovine serum, 100 U/mL penicillin, 100µg/mL streptomycin and 2 mM glutamine until confluence at 37°C and 10% CO_2. The following procedures refer to single cell culture flask with 75cm^2 surface. The medium was decanted from the cells. The cells

were washed with 10mL PBS and successively overlayed with 3mL Cell Dissociation Solution (CDS). The cells were incubated 1 minute at room temperature. Successively, CDS was removed and the cells were further incubated for 15 minutes at 37°C for dissociation. In the assay, 20µL of the solution containing the compounds to be tested was used. This solution did not contain more than 2.8% DMSO. For the dilution process, the compounds were diluted in 1/3 or 1/2 steps. To 20 µL of compound solution, 75µL of the RBL-cells were added, which were treated as follows: after dissociation, the cells were detached and warmed at 37°C in 10mL HAG-CM (20 mM Hepes, 125 mM NaCl, 5mM KCl, 1mM $CaCl_2$, 1mM $MgCl_2$, 0.5 mM glucose, 0.25% BSA). The cells were counted and centrifuged. The cell pellet was resuspended in preheated HAG-CM, and the cell density was adjusted to $2X10^6$ cells/mL. The cells were incubated at 37°C for 5 minutes. Per mL cell suspension, 2.7µL of a cytochalasin B-solution were added. The cells were incubated for a further 3 minutes at 37°C. 75µL of the cell suspension were added to 20µL compound solution leading to a volume of 95µL per well in a multititer plate (mtp). After incubation of the cells for 10 minutes at 37°C 10µL hrC5a per well are added. The cells were incubated for an additional 5 minutes at 37°C. The mtp was then transferred to ice and centrifuged for 3 minutes at 4°C with 250Xg. 75µL of the supernatant were added to 100µL substrate-solutions-D-glucosaminide in 42.5 mM β (2.7 mg/mL p-nitrophenyl-N-acetyl-Na acetate, pH 4.5). The mtp was incubated for further a 60 minutes at 37°C. 75µL of 0.4M glycine, pH 10.4, was added per well. The mtp was transferred into a reader and the absorption at 405nm

was measured. IC50-values were obtained following this equation: y=((A-D)/1+(x/C)B))+D.

7. The results of the assay are below:

Compound	Reference	Structure	C5aR antagonist activity [IC50, µM]	C5aR binding activity [IC50, µM]
1	Example 171 (US 5,387,671)	H-NMePhe-Lys-Pro-cha-Phe-DNMePhe-OH	> 19	*
2	Claimed compound	Hoo-Phe-Orn-Pro-hle-Pff-Phe-NH_2	0.039	*

* cited from US 5,387,671

8. These data show that Kawai example 171 performs very poorly as a C5a receptor antagonist. Kawai Example 171 has an activity in the functional assay of > 19 µM. From this value it is evident that this compound is essentially not active as a C5a receptor antagonist. In contrast, the claimed compound was a very effective C5a receptor antagonist with the difference in activity being more than 480-fold when compared to the Kawai example 171 compound. Based on the low activity shown above, a person skilled in the art would not be motivated to perform further experiments

with the Kawai example 171 compound and would specifically not have any motivation to derive any compounds with phenylalanine substituents, as in the claimed compound.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

25/04/08

[Heiko Hawlisch]